



PATENT  
2429-1-024

*DOC*  
*JPW*

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Eduardo PRIMO YUFERA *et al* Examiner: Not Assigned  
Serial No.: 10/630,367 Group Unit: 1632  
Filed: July 30, 2003  
For: ENTOMOPATHOGENIC MICROORGANISM SPORES CARRIER AND  
METHOD FOR CONTROLLING HARMFUL INSECTS

**CERTIFICATE OF MAILING UNDER 37 CFR 1.8**

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope with sufficient postage addressed to the COMMISSIONER OF PATENTS, P.O. Box 1450, Alexandria, VA 22313-1450 on May 8, 2006.

Lois A. Snure  
(Name of Depositor)

*Lois A. Snure* 5/8/06  
(Signature and Date)

**REQUEST FOR RECONSIDERATION OF PETITION UNDER 37 C.F.R. §1.47(a)**

Mail Stop Petition  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

Applicants previously filed a petition pursuant to the provisions of 37 C.F.R. §1.47(a) which provide that a single inventor may make application for patent in situations where a joint inventor cannot be found or reached after diligent effort. In relevant part, the provisions state that the oath or declaration may be signed by one inventor if accompanied by the instant petition setting forth proof of the pertinent facts together with the fee set forth in 37 C.F.R. §1.17(h) and the last known address of the non-signing inventor.

Applicants previously filed a Petition Pursuant to 37 C.F.R. §1.47(a) on January 23, 2006. On March 6, 2006, the Office of Petitions mailed a Decision Refusing Status under 37 C.F.R. §1.47(a). The Office of Petitions indicates that a Reply is due within two months thereby making a due date of May 8, 2006 (May 6 and 7 being a Saturday and Sunday respectively). The deficiencies in the Petition were identified as follows:

- (1) Applicants failed to show that inventor Pallares was ever presented with a copy of the application papers. Hence, to cure the deficiency, Applicants must demonstrate a bona fide attempt was made to present a copy of the application papers (specification, including claims, drawings, and oath or declaration) to the non-signing inventor Pallares; and
- (2) Applicants provided the last known mailing address of inventor Pallares in the Petition, however, the Declaration contains the mailing address of the Assignee. Hence, to cure the deficiency, Applicants must provide a Supplemental Declaration or Application Data Sheet providing the last known address of inventor Pallares.

Applicants cure these deficiencies by submitting herewith the following documents:

- (1) A letter dated April 18, 2006 to the last known address of inventor Pallares at Francisco Cubells Street with a Declaration and Assignment document as well as a copy of the application as filed;
- (2) A copy of the Official Acknowledgement of Receipt of the documents referenced in (1) above; and
- (3) A copy of the registered mail form as completed by the sender evidencing the mailing of the documents referenced in (1) above; and
- (4) A new Declaration intended for the signature of inventor Pallares containing his last known address.

It is believed that these documents cure the deficiencies of the Petition mailed January 26, 2006.

The last known address of the non-signing inventor, Juan Munoz Pallares, is  
C/Francisco Cubells, No. 14, bajo  
46011 Valencia  
SPAIN

Additional recent addresses of the non-signing inventor, Juan Munoz Pallares, include  
C/San Vicente Martir, No. 276  
46002 Valencia  
SPAIN

and

Paseo Juan de la Cierva, 6  
46900, Torrente  
Valencia  
SPAIN

In view of the foregoing demonstrating the inventor's recalcitrance, and joint inventor's diligent efforts at securing appropriate signature, Applicants request acceptance of the combined Declaration and Power of Attorney and Assignment documents as responsive to the Notice of Missing Parts mailed on August 23, 2005.

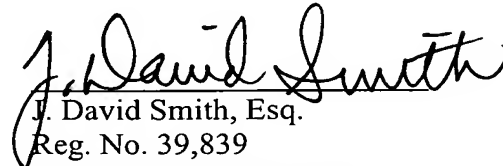
### **FEES**

No fees are believed necessary in connection with the present submission; however, should this be in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment or to credit any overage.

CONCLUSION

Acceptance of the petition is hereby requested, and grant of the petitioned relief is hereby sought.

Respectfully submitted,

  
J. David Smith, Esq.  
Reg. No. 39,839  
Attorney For Applicants

KLAUBER & JACKSON  
411 Hackensack Avenue  
Hackensack, New Jersey 07601  
(201) 487-5800



DOC. I

CENTRO DE APOYO A  
LA INNOVACIÓN, LA  
INVESTIGACIÓN Y LA  
TRANSFERENCIA DE  
TECNOLOGÍA  
Universidad Politécnica de  
Valencia

**CTT**

UNIVERSIDAD  
POLITECNICA  
DE VALENCIA

UNIVERSIDAD POLITECNICA DE VALENCIA  
C.T.T. INTERNAL REGISTER  
EXIT 00012  
18/04/2006 12:50  
Address to: Juan Muñoz Pallarés

**Juan Muñoz Pallarés**  
C/ FRANCISCO CUBELLS, 14 bajo  
46011 VALENCIA  
Valencia, 18<sup>th</sup> April 2006

Dear sir,

I am writing to you in relation to the application for national patent with file number **P200100266** bearing the title "**Carrier vehicle for spores of an entomopathogenic microorganism and method for combating harmful insects**" in which you are noted as one of the inventors.

I am notifying you that this University has decided to proceed with the international extension of that application, with all the expenses of the processing being borne by it.

By virtue of this letter, I inform you of your right to be noted as inventor in all documents making reference to the invention such as scientific papers, patents, etc. In this regard, we are enclosing herein a copy of the application as filed before the United States Patents Office, an Assignment document and a Power form for the present application. We ask you to sign said Power and Assignment document upon reviewing the patent application.

I ask you to return this documentation to the CTT once you have signed it, doing so by means of messenger to be paid for by the CTT, or by any other means that you consider appropriate, urgently.

For our part, we shall continue to inform you of any occurrences taking place during the processing and any modifications and final versions of the patent specification.

In the event that you wish to waive this right, the application shall in any case be processed in the same manner in order not to harm the rights of the other inventors, as well as those of this University and the Consejo Superior de Investigaciones Científicas, as the legal holders of the exploitation rights over the patent.

Thanking you in advance for your collaboration.

Yours faithfully,

Sila Durán  
Patents Manager  
CTT



### CERTIFICACION

Don José Joaquín Latas Díez,  
interprete jurado de lengua inglesa,  
certifica que la que antecede es traducción  
fiel y completa a lengua inglesa  
de un documento redactado en castellano.

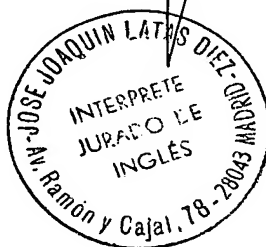
En Madrid, a 28 de abril de 2006

### CERTIFICATION

Mr. José Joaquín Latas Díez, sworn translator  
of the English language certifies, that the  
preceding translation is a true and complete  
version into English of a document written in  
Spanish.

Madrid, on the 28<sup>th</sup> April 2006

José Joaquín Latas Díez





UNIVERSIDAD POLITÉCNICA DE VALENCIA

REGISTRO INTERNO C.T.T.

**SALIDA 000673**

18/04/2006 12:50

Destino: JUAN MUÑOZ PALLARÉS



UNIVERSIDAD  
POLITECNICA  
DE VALENCIA

CENTRO DE APOYO  
A LA INNOVACIÓN,  
LA INVESTIGACIÓN  
Y LA TRANSFERENCIA  
DE TECNOLOGÍA  
Universidad Politécnica de Valencia



**Juan Muñoz Pallarés**

C/ Francisco Cubells nº 14, bajo  
46011, Valencia

Valencia, 18 de Abril de 2006.

Estimado señor:

Le escribo con relación a la solicitud de patente nacional con número de expediente **P200100266**, que lleva por título **"Vehículo portador de esporas de un microorganismo entomopatógeno y método para combatir insectos dañinos"**, en la que usted consta como uno de los inventores.

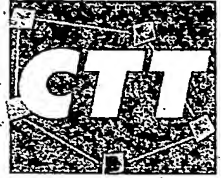
Le comunico que esta Universidad ha decidido continuar los trámites de la citada solicitud en distintos países, entre ellos Estados Unidos, corriendo de su cargo todos los gastos de la tramitación.

Por la presente carta, le informo de su derecho a constar como inventor en todos los documentos que hagan referencia a la invención como artículos científicos, patentes, etc. En este sentido, incluimos con la presente una copia de la solicitud de patente tal y como se ha presentado ante la Oficina de Patentes de Estados Unidos, un documento de Declaración y Poder para la presente solicitud. **Rogamos firme dicho documento de Declaración y Poder tras revisar la solicitud de patente.**

Le ruego proceda a la devolución al CTT de esta documentación una vez firmada, a través de mensajero a gastos pagado por el CTT o por cualquier otro medio que considere oportuno, a la mayor brevedad.

Por nuestra parte, le seguiremos informando de todas las incidencias que se produjesen en la tramitación, así como de las modificaciones y versiones definitivas de la memoria de patente.

En caso de que renunciase a este derecho; la solicitud se tramitará igualmente, a fin de no perjudicar el derecho de los demás inventores, así como los de esta Universidad Politécnica de Valencia. Centro de Apoyo a la Innovación, la Investigación y la Transferencia de Tecnología.- CTT. Edif. I1 y I2. Camino de Vera s/n. 46022. VALENCIA. Tel: 96 387 74 09. Fax 96 387 79 49.



Universidad y del Consejo Superior de Investigaciones Científicas, como titulares legales de los derechos de explotación sobre la patente.

Le agradezco de antemano su colaboración.

Atentamente,

Sila Durán  
Responsable de Patentes  
CTT



Attorney Docket No.: 2429-1-024

**DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below under my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**ENTOMOPATHOGENIC MICROORGANISM SPORES CARRIER AND METHOD FOR CONTROLLING HARMFUL INSECTS**

The Specification of which

- ☐ is attached hereto  
☒ was filed on July 30, 2003

I hereby state that I have reviewed and understand the contents of the above-identified Specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, 1.56(a):

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

APPLICATIONS NUMBER	PRIOR FOREIGN FILED APPLICATION(S)		
	COUNTRY	(MONTH/DAY/YYYY)	PRIORITY CLAIMED
P 090190266	Spain	January 30, 2001	YES

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER(S)	FILING DATE (MM/DD/YYYY)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or §365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application No.	PCT Parent Number	Parent Filing (MM/DD/YYYY)	Parent Patent Number (if applicable)
	PCT/ES02/00043	January 30, 2002	

**BEST AVAILABLE COPY**

Attorney Docket No.: 2429-1-024

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from UNGRIA PAENTES Y MARCAS, S.A. as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

I hereby appoint as my attorneys or agents the registered persons identified under

Customer No. 23565

of the law firm of Klauber & Jackson, said attorneys or agents with full power of substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Please address all correspondence regarding this application to Customer No. 23565.

DAVID A. JACKSON, ESQ.  
KLAUBER & JACKSON  
411 HACKENSACK AVENUE  
HACKENSACK, NEW JERSEY 07601

Direct all telephone calls to David A. Jackson at (201) 487-5800.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF FIRST JOINT INVENTOR: Eduardo PRIMO YUFERA

COUNTRY OF CITIZENSHIP: Spain

FULL RESIDENCE ADDRESS: Instituto de Tecnología Química  
Consejo Superior de Investigaciones Científicas  
Universidad Politécnica de Valencia  
Los Naranjos, s/n  
E-46022 VALENCIA  
Spain

FULL POST OFFICE ADDRESS: SAME AS ABOVE

SIGNATURE OF INVENTOR \_\_\_\_\_

DATE \_\_\_\_\_

**BEST AVAILABLE COPY**

Attorney Docket No.: 2429-1-024

FULL NAME OF SECOND JOINT INVENTOR: Mohamed IBRAHIM FAHMY

COUNTRY OF CITIZENSHIP: Spain

FULL RESIDENCE ADDRESS: Instituto de Tecnología Química  
Consejo Superior de Investigaciones Científicas  
Universidad Politécnica de Valencia  
Los Naranjos, s/n  
E-46022 VALENCIA  
Spain

FULL POST OFFICE ADDRESS: SAME AS ABOVE

SIGNATURE OF INVENTOR \_\_\_\_\_


DATE \_\_\_\_\_

FULL NAME OF THIRD JOINT INVENTOR: Juan MUÑOZ PALLARES

COUNTRY OF CITIZENSHIP: Spain

FULL RESIDENCE ADDRESS: Francisco Cubells, 14 bajo  
E-46011 VALENCIA  
Spain

FULL POST OFFICE ADDRESS: SAME AS ABOVE

SIGNATURE OF INVENTOR  \_\_\_\_\_

DATE \_\_\_\_\_

FULL NAME OF FOURTH JOINT INVENTOR: Pilar MOYA SANZ

COUNTRY OF CITIZENSHIP: Spain

FULL RESIDENCE ADDRESS: Instituto de Tecnología Química  
Consejo Superior de Investigaciones Científicas  
Universidad Politécnica de Valencia  
Los Naranjos, s/n  
E-46022 VALENCIA  
Spain

FULL POST OFFICE ADDRESS: SAME AS ABOVE

SIGNATURE OF INVENTOR \_\_\_\_\_

DATE \_\_\_\_\_

U 00015622312

Mod. 35 PLUS - 1E

Aviso de Recibo  
Avis de Rebuda  
CERTIFICADO  
CERTIFICAT

DESTINATARIO DEL ENVÍO / DESTINATARI DE L'ENVIAMENT

JUAN MUÑOZ PALLARES

C/ FRANCISCO GIBERES N°19  
46011 VALENCIA

46011 VALENCIA

VALENCIA

Cumplimentar en MAYÚSCULAS  
Emplenar en MAJÚSCULES

Devolver a:  
Retornar a:

D/D\*  
En/Na

Domicilio  
Domicili

C.P. y Población  
C.P. i Població

Provincia  
Província

REMITENTE DEL ENVÍO / REMITENT DE L'ENVIAMENT

UNIVERSIDAD POLITÉCNICA DE VALENCIA  
D. S. LA DURAN

CIT - CAMINO DE VERA, S/N  
EDIF. I1 y I2

46022 VALENCIA

VALENCIA

CORREOS



ATENCIÓN NO SOBREPASAR POR ABAJO ESTE LÍMITE • ATENCIÓ: NO SOBREPASSAR PER SOTA D AQUEST LÍMIT



DOC. 2

Form 35 PLUS 1E ACKNOWLEDGEMENT OF RECEIPT	
<i>Spanish Mail Service</i>	
REGISTERED	
<p>CU 00015622312</p> <p>Addressee: .... JUAN MUÑOZ PALLARES .....</p> <p>Address: C/ FRANCISCO CUBELSS, ..... No. 14 ..... Floor .....</p> <p>Post Code ..... 46011 ..... Town or city: VALENCIA</p> <p>FILL IN CAPITAL LETTERS</p>	<p>Sent by:</p> <p>Return to:</p> <p>Address:....Ms. Sila Durán.....</p> <p>UNIVERSIDAD POLITÉCNICA DE VALENCIA..... No. .... Floor CTT Camino de Vera, S/N.....</p> <p>Post Code . ...46022.....</p> <p>Town or city: Valencia.....</p> <p>.....</p> <p>Province:..VALENCIA.....</p>

BACK SIDE:

CU 00015622312

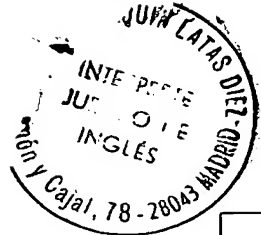
receipt

The undersigned declares that the letter was duly delivered

NAME AND FAMILY NAMED OF RECEIVER:  
GERARDO ROMERO POLO

IDENTITY CARD NUMBER OF RECEIVER:

53205797-N



### CERTIFICACION

Don José Joaquín Latas Díez,  
interprete jurado de lengua inglesa,  
certifica que la que antecede es traducción  
fiel y completa a lengua inglesa  
de un documento redactado en castellano.

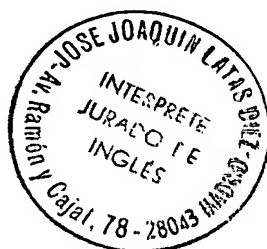
En Madrid, a 28 de abril de 2006

### CERTIFICATION

Mr. José Joaquín Latas Díez, sworn translator  
of the English language certifies, that the  
preceding translation is a true and complete  
version into English of a document written in  
Spanish.

Madrid, on the 28<sup>th</sup> April 2006

José Joaquín Latas Díez





# CORREOS

CU6001452312

CIF: A83052407 OFICINA: 4614394 - VALENCIA SUC 12

ESPACIO PARA  
CÓDIGO DE BARRAS  
ESPAL A  
CODIG DE BARRES

M-11

INFORMACIÓN 902 197 197 INTERNET WWW.CORREOS.ES

REMITENTE/REMITENT

D. UNIVERSIDAD POLITÉCNICA DE VALENCIA NIF Q-4618007-B

DESTINATARIO/DESTINATARI

D. JUAN MUÑOZ PALLARES

C. FRANCISCO CUBÉLLS

Población VALENCIA

C. P. 46011 Prov. VALENCIA

nº 14 piso BAJA  
pis

País ESPAÑA

Carta Certificada Urgente

Fecha: 18/04/2006 Aviso de recibo: C

Peso: 146 gr.

Hora: 13:24

Importe: 5,29 €

Sello de fechas o validación mecánica  
Segell de dates o validació mecànica

ESPACIO A RELLENAR POR CORREOS

ESPAU A OMLLR PER CORREOS

MODALIDAD

CLASE

☐ Carta ☐ Paquete Postal

☐ Sacas M ☐ Paquete Azul

☐ Urgente ☐ Urgent

☐ Aviso de Recibo ☐ Aviso de Recib

☐ Reembolso/Reemborsament

☐ Asegurada/Asegurada

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☐ Asegurada/Asegurada

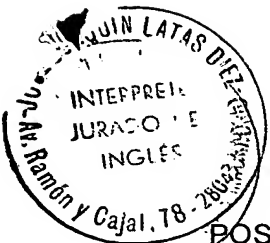
☐ Reembolso/Reemborsament

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☐ Asegurada/Asegurada

Vascom, S.L. - 2005V



**DOC. 3**

**POST SERVICE**  
CU0001522312

**CIF:** A83052407 **OFFICE:** 4614394 - VALENCIA SUC 12

**SENDER:**

UNIVERSIDAD POLITÉCNICA DE VALENCIA / NIF: Q-4618002-B

**RECEIVER:**

Mr. JUAN MUÑOZ PALLARES

C/ FRANCISCO CUBELLS NO. 14, FLOOR: GROUND FLOOR

**CITY:** VALENCIA

**POST CODE:** 46011 **PROVINCE:** VALENCIA **COUNTRY:** SPAIN

**URGENT REGISTERED LETTER**

**DATE:** 18/04/2006 **ACKNOWLEDGMENT OF RECEIPT:** 0

**WEIGHT:** 146 g

**TIME:** 13:24

**COST:** EUROS 5.29





### CERTIFICACION

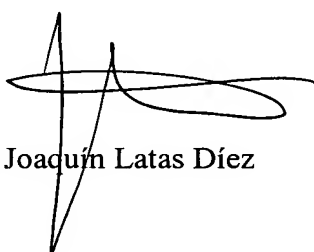
Don José Joaquín Latas Díez,  
interprete jurado de lengua inglesa,  
certifica que la que antecede es traducción  
fiel y completa a lengua inglesa  
de un documento redactado en castellano.

En Madrid, a 28 de abril de 2006

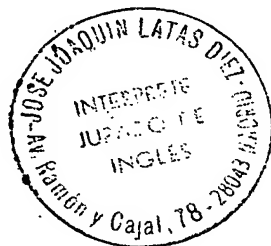
### CERTIFICATION

Mr. José Joaquín Latas Díez, sworn translator  
of the English language certifies, that the  
preceding translation is a true and complete  
version into English of a document written in  
Spanish.

Madrid, on the 28<sup>th</sup> April 2006



José Joaquín Latas Díez



EXPRESS MAIL NO. EV 354827637

DATE OF DEPOSIT: JULY 30, 2003

PTO/SB/13/PCT (06-03)

Approved for use through 07/31/2003. OMB 0651-0032  
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

## REQUEST FOR FILING A CONTINUATION OR DIVISION OF AN INTERNATIONAL APPLICATION

DOCKET NUMBER	ANTICIPATION CLASSIFICATION OF THIS APPLICATION		PRIOR APPLICATION EXAMINER	ART UNIT
2429-1-024	CLASS	SUBCLASS		

## Address to:

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

This is a request for filing a ☒ continuation ☐ divisional application under 37 CFR 1.53(b) of pending prior international application Number PCT ES02/00043, filed on Jan. 30, 2002 entitled ENTOMOPATHOGENIC MICROORGANISM SPORES CARRIER AND METHOD FOR CONTROLLING HARMFUL INSECTS which designated the United States.

Note: 37 CFR 1.53(d) cannot be used to file a continuation or divisional application of an international application which has not entered the national stage.

CLAIMS	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
	TOTAL CLAIMS (37 CFR 1.16(c))	23 - 20 =	3	x \$ 18. =	\$ 54.00
	INDEPENDENT CLAIMS (37 CFR 1.16(b))	1 - 3 =	0	x \$----- =	
	MULTIPLE DEPENDENT CLAIMS (if applicable) (37 CFR 1.16(d))			x \$----- =	
				BASIC FEE (37 CFR 1.16(a))	750.00
				Total of above Calculations =	804.00
	Reduction by 50% for filing small entity (Note 37 CFR 1.27).				402.00
	Total =				402.00

- Enclosed are the specification, claims and drawing(s).
- ☒ Applicant claims small entity status. See 37 CFR 1.27.
- ☐ The Director is hereby authorized to charge any fees which may be required under 37 CFR 1.16 and 1.17, or credit any overpayment of Deposit Account No. 11-1153. A duplicate copy of this sheet is enclosed.
- ☐ A check in the amount of \$ \_\_\_\_\_ is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☐ Application Data Sheet is enclosed. See 37 CFR 1.76.
- ☐ Amend the specification by inserting before the first line the sentence: "This application is a ☐ continuation ☐ division of international application number PCT \_\_\_\_\_, filed \_\_\_\_\_, (status, abandoned, pending, etc.)."

[Page 1 of 2]

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

**REQUEST FOR FILING A CONTINUATION OR DIVISION OF AN INTERNATIONAL APPLICATION**

8. ☒ A declaration under CFR 1.63 is enclosed. Unexecuted
9. ☒ Priority of foreign application number 200100266, filed on January 30, 2001  
in Spain is claimed under 35 U.S.C. 119(a)-(d).
- ☐ The certified copy is enclosed.
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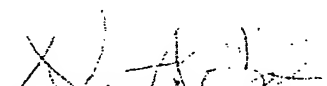
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July 30, 2003

Date

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Registration Number, if applicable

- ☐ Inventor(s)/Applicant(s)
- ☐ Assignee of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).
- ☒ Attorney or agent of record
- ☐ Filed under 37 CFR 1.34(a)  
Registration number if acting under 37 CFR 1.34(a) \_\_\_\_\_

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below\*.

☐ \*Total of \_\_\_\_\_ forms are submitted.

TITLE  
ENTOMOPATHOGENIC MICROORGANISM SPORES CARRIER AND METHOD  
FOR COTROLLING HARMFUL INSECTS

RELATED APPLICATIONS

[0001] The present application is a Continuation of co-pending PCT Application No. PCT/ES02/00043, filed January 30, 2002 which in turn, claims priority from Spanish Application Serial No. 200100266, filed on January 30, 2001. Applicants claim the benefits of 35 U.S.C. §120 as to the PCT application and priority under 35 U.S.C. §119 as to said Spanish application, and the entire disclosures of both applications are incorporated herein in their entireties.

TECHNICAL FIELD OF THE INVENTION

[0002] The present invention is set within the agricultural sector, particularly in the field of process for combating insects. More specifically, it refers to a carrier vehicle of spores of at least one entomopathogenic microorganism adsorbed on a granular porous support or in powder form; to a device which includes said composition or said vehicle; to an incorporated attractant semiochemical component and to a method for combating insects by means of the use of said device.

BACKGROUND OF THE INVENTION

[0003] The use of insecticides in controlling plagues of insects presents problems such as toxicity; the lack of selectivity which leads to the destruction of beneficial insects or of natural predators of the plague it is sought to combat; and the resistance developed by the insects which causes an increase in the dose needed to maintain its efficacy.

**[0004]** The use of semiochemical substances has developed substantially and, in particular, techniques such as surveillance in order to anticipate the appearance of plagues and extinguish them, sexual confusion in order to prevent reproduction of the insects, and mass capture in order to decrease the population of them.

**[0005]** Semiochemicals display low toxicity and very high selectivity, since they act on a single species of insect. Also, they do not provoke any resistance among insects or contaminant effects for the environment.

**[0006]** As well as the use of semiochemicals in a process for combating insects, the entomopathogenic properties of certain types of microorganisms, such as fungi, can also be exploited. These properties are known, but their effectiveness in biological control of insect plagues depends largely on their method of application.

**[0007]** For the specific and directed application of entomopathogenic microorganisms, supports are needed for the spores that will keep them viable and without germinating for long periods of time in the field and, moreover, combining them with specific semiochemicals that will attract the particular species to be combated, towards the contaminant support.

**[0008]** For the use of semiochemicals to be effective, it is necessary to have physical supports complying with requisites such as:

- providing an adequate emission rate of the semiochemical,
- permitting prolonged duration of the emission,
- avoiding degradation of the semiochemical,

- not producing contaminant waste, and
- to be economical and to permit easy application of the semiochemical.

[0009] Of the emitter supports existing on the market, such as, for example, rubber septa, polyethylene pipes, porous plastic sheets, etc., none of them complies with the stated requisites.

[0010] Moreover, the methods so far used for the application of entomopathogenic microorganisms present problems of survival which diminish their efficacy.

[0011] Application WO-9101736 claims a gel of natural polymers to which attractant entomopathogens, feeding stimulants and protectors against UV are incorporated. It also claims several forms of gelling the polymers by means of metal cations and the insects ingest the entomopathogen with the gel.

[0012] The differences with the present process are that what is claimed is the adsorption of entomopathogenic fungi spores in adsorbent materials selected among silicates, silicoaluminates, phosphoaluminates and ion exchange resins, which further incorporate the insect-specific volatile attractant compounds (pheromones and other natural or synthetic attractants) in such a way that a slow, controlled and lasting emission of the same takes place (Patent UPV No. P 9701077) and they maintain a suitable degree of humidity for the survival of the spores.

[0013] WO-A-9208355 claims a process for drying microbial cultures, mixing them with a carrier like those used for formulating insecticides for dusting and drying, them with air. Its application is by means of coating

seeds with microbial dust and is of no use for protecting aerial parts.

**[0014]** Application WO-9211356 claims some particular strains of entomopathogenic fungi. It also claims a formulation wherein the spores are incorporated into an emulsified bait containing cotton seed flour (proteins and carbohydrates), extracts of parts of cotton plants, oil and an emulgent, all these as a "feeding stimulant". This bait is sprayed in liquid form or it is added to a solid carrier in powder form, in the usual form of insecticide formulations and is applied as a wettable powder or in dusting.

**[0015]** WO-9324013 claims a storage chamber for entomopathogenic fungi for their conservation until they are used for controlling insects such as cockroaches, flies, ants and plagues of larvae, plagues on lawns and caterpillars. In these chambers, the temperature, humidity and oxygen are regulated. An emulgent can also be added for applying the fungus in aqueous suspension or in powder form. It cites two patents for cockroach traps.

**[0016]** WO-9510597 claims a formulation of entomopathogenic fungi wherein the conidia are suspended in a mineral oil. This formulation is used for being applied by spraying on crops. It can be made emulsionable for dispersion in water by the addition of an emulgent or adding an inert carrier to it for its application in the form of a suspendible powder as is usual in insecticide formulations.

**[0017]** EP-A-0406103 claims the culture of an entomopathogenic fungus on an inert solid support such as montmorillonite or atapulgite and the use of the resulting mass for spraying against plagues or for

fermenting in the soil under sporulation conditions.

[0018] The use of adsorbent materials as controlled rate emitters of insect attractants - generally sexual pheromones - is described WO-A-9944420-A and WO-A-0000446. Nevertheless, the process described in these patent applications does not include the use of an entomopathogenic agent.

[0019] As is revealed from the above paragraphs, both the actual existing physical supports and, in general, the methods for combating insects based on the use of entomopathogenic microorganisms still display serious deficiencies in several basic aspects, such as the period of duration of the composition or vehicle used and the demonstrated efficacy, for which reason there exists a demand for the development of new insect control systems.

#### **DESCRIPTION OF THE INVENTION**

[0020] The present invention aims to overcome the drawbacks of the state of the art by means of a carrier vehicle for spores of at least one entomopathogenic microorganism, consisting of an adsorbent support selected among a granular form, a powder form and mixtures of them, capable of retaining the spores and maintaining their viability and a attractant semiochemical component, wherein:

the adsorbent support is a material selected among silicates, silicoaluminates, phosphoaluminates, ion exchange resins and combinations of them;

the spores are adsorbed onto the mineral support;

and the semiochemical component, selected among attractant semiochemical substances of insects susceptible of suffering the entomopathogenic effects of the spores, is also adsorbed on that support or on



another analogous support.

**[0021]** The advantage of this invention is that it combines three different effects:

- a) The fixing of the biological material on a support which adsorbs it on its surface in an adequate way for contaminating insects by contact and for maintaining its viability during a long period of time.
- b) This support maintains by adsorption a degree of humidity that is adequate for preventing the biological material from drying out yet is insufficient for its germination, giving a prolonged life.
- c) The same or another support, or a mixture of them, adsorbs the attractant producing a controlled and lasting emission thereof and ensuring the specificity.
- d) An adhesive fixes the adsorbent support, in powder or granular form, on a solid surface (plates, spheres, etc.). This adhesive can be an organic polymer or an aqueous gel which contributes to maintain a constant and regulated supply of humidity to the adsorbent support located thereon.
- e) The local form of application is more specific and ecological than general spraying.
- f) The carrier support of spores and/or of attractants is adhered to a solid surface (plates, spheres, etc.) by means of a natural or synthetic polymeric adhesive, and the device is located in the field in the usual way for traps.

**[0022]** A first additional object of the present invention is a composition for combating insects which incorporates that vehicle comprising spores of at least

one entomopathogenic microorganism, at least one adsorbent support selected from a granular form or in powder form and an attractant semiochemical component.

**[0023]** A second additional object of the present invention is a device for combating insects, characterized in that it comprises a receptacle comprising, in a way that is accessible for insects, a carrier vehicle of spores of at least one entomopathogenic microorganism, at least one adsorbent support in granular or powder form, and an attractant semiochemical component.

**[0024]** A third additional object of the present invention is a method for combating insects by means of infection of them with spores of at least one entomopathogenic microorganism, characterized in that an efficacious quantity of the vehicle or of the composition obtained, according to the invention, is made available to the insects.

**[0025]** The entomopathogenic microorganism can be any fungus or bacterium capable of contaminating the insects. In a preferred manner, said microorganism is a fungus, for example *Metarhizium anisopliae*, *Paecilomyces fumosoroseus*, *Beauveria bassiana*, etc. The spores of the entomopathogenic microorganism are present in a quantity between  $1 \times 10^3$  and  $1 \times 10^{12}$  spores per gram of adsorbent support.

**[0026]** The support is a natural or synthetic adsorbent material selected from among silicates, silicoaluminates, phosphoaluminates, ion exchange resins, or any combination of them. In a preferred manner, said support is a zeolite and more preferably still it is a sepiolite.

**[0027]** The size of the structural channels of the adsorbent support must be adequate for housing the spores yet preventing them from penetrating to the interior of the structure of the support when exposed and accessible to insects. This size of particle lies between 230-450 nm, preferably between 240 nm and 420 nm (figure 1). The quantity of adsorbent support used is between 50 and 60 mg per cm<sup>3</sup>.

**[0028]** The device of the present invention can furthermore include an intermediate base for fixing the adsorbent support on a flat or curved solid surface. This intermediate base can be any organic adherent polymer, such as for example an elastomeric adhesive, or an emulsion of them, or an aqueous adherent gel obtained with one or more natural or synthetic gelling agents, such as for example agar, alginates or other polymers of algae and fungi, carboxymethylcellulose, crystalline cellulose, quitosanes and derivatives, methylcellulose and methylbutylcellulose.

**[0029]** When this adherent intermediate base is an aqueous gel, a wetting agent can also be added to it, such as sorbitol, glycerol, manitol, xylitol and combinations thereof. Desiccation is thereby avoided and this intermediate base also helps to maintain the humidity of the adsorbent support in powder or granular form for the spores.

**[0030]** In a preferred embodiment, methylbutylcellulose is used. In another preferred embodiment, a mixture is used of carboxymethylcellulose (CMC) and methylcellulose (MC) in a proportion of CMC and MC of between 5 and 40 % by weight. The moistening agent is selected from among one or more polyalcohols, in a preferred way it is selected from among sorbitol, glycerol, manitol, xylitol

and combinations thereof. In a still more preferred way, sorbitol or glycerol is used.

**[0031]** The quantity of said moistening agent in the device is between 20 % and 96 % by dry weight. It is preferably in a proportion of from 25 % to 85 % by dry weight of the device.

**[0032]** The attractant semiochemical component is specific for the species being dealt with and can be a pheromone or other natural or synthetic attractant that produces an adequate response. Specific examples of semiochemicals are trimedlure and 1-4-tetramethylenediamine, specific attractants of *Ceratitis capitata*, or methyl-eugenol, an attractant of *Bactrocera dorsalis*. The semiochemical component is present in the adsorbent support in a proportion between 0.005 - 1.0 gram per gram of adsorbent support, preferably in a proportion between 0.02-0.7 grams per gram of said adsorbent support.

**[0033]** The adsorbent support of the present invention can furthermore comprise an oil component selected among mineral oils, vegetable oils, animal oils and mixtures thereof, which contributes to fixing the attractant semiochemical component and protecting the spores. The function of the oil component is to help in the retention of the semiochemical and its slow and controlled emission and to maintain the spores in an oily medium in order to increase their protection and extend their life-time. Said oil component is present in the vehicle by an amount between 20 % and 75 %.

**[0034]** The device of the present invention can adopt various forms according to the arrangement of its components, thereby ensuring the maximum duration of the

attraction and contamination of the insect. So, the surface coated with the support can be flat (plates of different dimensions, folded or unfolded) or curved (spheres imitating fruits, cones, cylinders and other shapes).

**[0035]** In a first form, an adsorbent support containing the spores and the semiochemical component with or without oil is adhered to a solid surface by means of organic adhesives or aqueous gels.

**[0036]** In a second form, the spores and the semiochemical component are adsorbed onto different adsorbent supports. The mixture of the two supports is adhered to a solid surface by means of organic adhesives or aqueous gels.

**[0037]** In a third form, the adsorbent support containing the semiochemical component is incorporated into the adhesive base layer and the adsorbent support containing the spores is spread on the surface.

**[0038]** In a fourth form, the adsorbent support containing the semiochemical component is in the form of a pill, located in the centre of the adhesive base layer and surrounded by the adsorbent support containing the spores.

**[0039]** The vehicle of the present invention can include a UV ray photoprotector in its composition.

**[0040]** For its transportation and use, the vehicle of the present invention is located on an object, for example, plates or spheres, the surface of which contains an adherent component and the adsorbent. These plates are located in the field underneath the traps, which protect

them from the sun and rain.

**[0041]** An additional object of the present invention is a composition that includes a carrier vehicle for spores of at least one entomopathogenic microorganism adsorbed on a support, as specified earlier, the application of which can be done in a suspension of the vehicle in a fluid, such as water for example, or by dusting, in a manner analogous to insecticide formulations.

**[0042]** A second additional object of the present invention is a device for combating insects, comprising a receptacle which, in a manner accessible for the insects, comprises a vehicle or a composition which incorporates spores of at least one entomopathogenic microorganism adsorbed on an adsorbent support, as has been specified earlier.

**[0043]** By means of the present invention, a long period of activity of the spores is achieved since the device maintains the necessary humidity in the adsorbent material for their survival and permits the degree of humidity to be maintained for prolonged periods, of the order of 2 to 5 months of exposure in the field.

**[0044]** Another advantage over the prior art is the selective effect that is achieved, thanks to the use of an attractant semiochemical, specific to the species to be dealt with.

**[0045]** Another advantage of the present invention consists of the use of particles of adsorbent material as support for the spores, which grants efficacy to the method of combating insects since it ensures its contact with the insect and contamination of it, as well as

acting as an attractant vehicle and so that the humidity can reach the spores.

### EXAMPLES

[0046] The following examples serve to illustrate the different aspects of the invention.

#### EXAMPLE 1

[0047] An adherent layer of 2 mm of polyisobutylene is deposited on a plastic plate, and on this another layer of MCM-41 zeolite carrier of adsorbed spores of *Metarhizium anisopliae* suspended in mineral oil is deposited. The zeolitic support is also impregnated with specific attractants of *Ceratitis capitata*, for example, with trimedlure or 2-4-tetramethylenediamine in a proportion of 0.5 and 0.005 g per gram of zeolite.

[0048] Table 1 shows the result obtained with the spore carrier vehicle, exposed in the field for three months. In particular, the effect of ageing is shown on the physical and biological properties of the attractant-contaminant plates of *Ceratitis capitata*, along with the results of its action on the insects.

[0049]

TABLE 1

Time (days)	Power of Attraction (%) <sup>a)</sup> (mean $\pm$ SD)	Mortality (%) <sup>b)</sup> (mean $\pm$ SD)	Loss of humidity (%)
0	2.10 $\pm$ 1.5	15.0 $\pm$ 0.9	0
15	8.2 $\pm$ 0.5	45.8 $\pm$ 1.6	35.4 $\pm$ 1.1
30	30.2 $\pm$ 2.6	68.4 $\pm$ 2.7	45.1 $\pm$ 2.7
45	37.4 $\pm$ 2.1	70.3 $\pm$ 2.7	59.7 $\pm$ 3.6
60	46.7 $\pm$ 1.7	76.4 $\pm$ 3.4	65.4 $\pm$ 2.6
75	40.9 $\pm$ 0.9	70.8 $\pm$ 3.2	69.3 $\pm$ 1.8
90	27.5 $\pm$ 2.5	67.6 $\pm$ 1.8	72.3 $\pm$ 3.1

<sup>a)</sup> Mean count of males alighting on the plate, every 5 minutes, for 3 hours.

<sup>b)</sup> Number of dead flies as a result of mycosis following a 24 hour exposure period to the plate.

EXAMPLE 2

[0050] A plate is prepared with a gelled adherent base of methybutylcellulose in a proportion that can vary from 10 to 45 %. Glycerol is added as a moistening agent in a proportion of 20 to 75 % by dry weight. Deposited on the gel is a layer of sepiolite of particle size between 240-420 nm, which is impregnated with spores of *Paecilomyces fumosoroseus*, suspended in mineral oil. The attractant semiochemical used is methyl-eugenol, an attractant of *Bactrocera dorsalis*, which is adsorbed on another portion of sepiolite in a proportion between of 0.8 gram of sepiolite and is compacted to form a pill, which is partially submerged in the centre of the plate carrying the gel component.



[0051] The plates are located in the field underneath the traps, which protect them from the sun and rain.

[0052] Table 2 shows the effect of ageing in the physical and biological properties of the attractant-contaminant plates of *Bactrocera dorsalis*, along with the results of its action on the insects.

[0053]

TABLE 2

Time (days)	Power of Attraction (%) <sup>a)</sup> (mean $\pm$ SD)	Mortality (%) <sup>b)</sup> (mean $\pm$ SD)	Loss of humidity (%)
0	6.10 $\pm$ 5	30.0 $\pm$ 0.7	0
15	11.2 $\pm$ 1.3	40.3 $\pm$ 1.1	30.1 $\pm$ 1.6
30	35.4 $\pm$ 1.9	78.0 $\pm$ 2.8	45.1 $\pm$ 2.7
45	49.8 $\pm$ 3.4	84.7 $\pm$ 3.4	43.4 $\pm$ 2.4
60	55.9 $\pm$ 2.4	90.3 $\pm$ 2.1	46.3 $\pm$ 2.6
75	50.2 $\pm$ 1.3	85.7 $\pm$ 1.4	50.1 $\pm$ 2.2
90	38.3 $\pm$ 2.2	70.4 $\pm$ 0.9	62.3 $\pm$ 1.3

<sup>a)</sup> Mean count of males alighting on the plate, every 5 minutes, for 3 hours.

<sup>b)</sup> Number of dead flies as a result of mycosis following a 24 hour exposure period to the plate.

## CLAIMS

1. A carrier vehicle of spores of at least one entomopathogenic microorganism on an adsorbent support selected among a granular form, a powder form and mixtures thereof, and of an attractant semiochemical component, characterized in that:

the natural or synthetic adsorbent support is a material selected from among silicates, silicoaluminates, phosphoaluminates, ion exchange resins and combinations of them;

the spores are adsorbed onto the mineral support and located on the surface in a way that is accessible to the attracted insects;

and the semiochemical component is selected from among semiochemical substances specifically attractant of the species of insect it is wished to combat and is adsorbed on an adsorbent support.

2. A vehicle according to claim 1, characterized in that it furthermore comprises an adherent intermediate base for retaining the adsorbent powder and preventing its dispersion. This base can be a viscous polymer such as an elastomer insoluble in water or an aqueous gel with a moistening agent. This adsorbent intermediate base is applied on plates, spheres or other solid shapes for being located in the field.

3. A carrier vehicle of spores according to claim 1, characterized in that the spores are suspended in an oil component selected among mineral oils, vegetable oils, animal oils and mixtures of them, and adsorbed on the adsorbent support.

4. A vehicle according to claim 2, characterized in that the attractant semiochemical component is adsorbed

on the adsorbent support and said support, coated with the spores, is spread on the intermediate adherent.

5. A vehicle according to claim 2, characterized in that:

the semiochemical component is adsorbed on a natural or synthetic support selected among a granular form, a powder form and mixtures thereof, and selected from among silicates, silicoaluminates, phosphoaluminates, ion exchange resins and combinations of them, and this support can be the same as or different from the spore carrier.

6. A vehicle according to claim 3, characterized in that the spores are suspended in an oil component selected among mineral oils, vegetable oils, animal oils and mixtures of them.

7. A vehicle according to claim 5, characterized in that:

the semiochemical component is adsorbed on the adsorbent support, the same as or different from the spore carrier.

the adsorbent support together with the semiochemical is compacted in pills, which are surrounded by the carrier support for spores. The two adsorbent supports can be the same or different.

8. A vehicle according to claim 3, characterized in that: the spores are suspended in an oil component selected among mineral oils, vegetable oils, animal oils and mixtures of them.

9. A vehicle according to claim 2, characterized in that: the intermediate adhesive layer is selected among organic polymeric adhesives or is an aqueous gel of

natural or synthetic polymers.

10. A vehicle according to claim 9, characterized in that: the adherent layer is formed from organic adhesives or from gels, the gelling substance of which is selected among natural or synthetic hydrophilic polymers such as carboxymethylcellulose, crystalline cellulose, carboxymethylchitosane, methylcellulose, methylbutylcellulose, quitosanes, polymers of algae and plants and combinations thereof.

11. A vehicle according to claim 2, characterized in that the moistening agent consists of one or more polyalcohols.

12. A vehicle according to claim 11, characterized in that the moistening agent is selected among sorbitol, glycerol, manitol, xylitol and combinations thereof.

13. A vehicle according to claim 2, characterized in that the moistening agent is present in a quantity between 20 % and 95 % of the dry weight of the gelling agent.

14. A vehicle according to claim 1, characterized in that the semiochemical component comprises at least one pheromone or a synthetic or natural attractant, specific for the species is it wished to combat.

15. A vehicle according to claim 1, characterized in that the semiochemical component is present in the adsorbent support in a quantity between 0.005 and 1 g per gram.

16. A vehicle according to claim 5, characterized in that the semiochemical component is present in the second

adsorbent support in a quantity between 0.0005 and 1 g per gram of said second adsorbent support.

17. A vehicle according to claim 1, characterized in that the entomopathogenic microorganism can be any fungus or bacterium capable of contaminating the insects.

18. A vehicle according to claim 1, characterized in that the spores of the entomopathogenic microorganism are present in a quantity between  $1 \times 10^3$  -  $1 \times 10^{12}$  spores per gram of adsorbent support.

19. A vehicle according to claim 3, characterized in that the oil component is present in a quantity between 30-75 % per gram of adsorbent support.

20. A composition for combating insects which contains a vehicle as defined according to claim 1.

21. A device for combating insects, characterized in that it comprises a receptacle containing, in a form accessible for the insects, a vehicle defined according to claim 1.

22. A device for combating insects, characterized in that it comprises a receptacle containing, in a form accessible for the insects, a composition defined in claim 20.

23. A method for combating insects by means of infection of the insects with spores of microorganisms, characterized in that an efficacious quantity of spores is made available to the insects, according to any of claims 1.

### ABSTRACT

The patent describes a method of selective application of entomopathogenic fungi, characterized by employing an attractant-contaminant device in which the spores of said fungus are fixed on an adsorbent material; this same adsorbent material or another, depending on the case, incorporates a specific attractant and is located on an adherent material. This adherent material can, in certain cases, incorporate a gelling agent and different additives, which maintain the adequate level of humidity for the survival of the spores.

Standing out among the advantages of this ecological method of application of entomopathogenic fungi is the selectivity resulting from the use of specific attractants and the long duration of the attractant-contaminant effect thanks to the use of the controlled rate emitter (adsorbent substance) and to the fact that a greater persistence of the spores is achieved with control of the humidity.